Report for 2004MN48B: Phyto-enhanced Remediation: A Wetland Treatment System for Surface Water Protection

- Articles in Refereed Scientific Journals:
  - DeJournett, T., Fritsch, J., McNeil, K., and Arnold, B., 2005. Preparation of 14C-cis-1,2-dichloroethylene from 14C-trichloroethylene using a cobalt porphyrin catalyst. Journal of Labelled Compounds and Radiopharmeceuticals, 48(5): p. 353-357.
  - DeJournett, T.D; Arnold, W.A.; LaPara, T.M., 2005. The Effect of Vegetation on Methanotrophic Bacterial Populations in a Constructed Wetland, Applied Soil Ecology, in review.
- Other Publications:
  - Characterization of methanotrophic bacterial populations in the rhizosphere of emergent wetland plants. Poster N-096 American Society for Microbiology 104th Annual Meeting, New Orleans, LA, May 23-27 2004.
  - Stimulation of methanotrophic bacteria in a wetland treatment system. Environmental Engineering Seminar, University of Missouri-Rolla November 7, 2003.
  - Phyto-Enhanced Remediation: A constructed wetland for removal of chlorinated ethylenes from groundwater Poster Presentation, Frontiers in Assessment Methods for the Envrironment (FAME), Minneapolis, MN August 10-13 2003.
  - Phyto-Enhanced Remediation: A constructed wetland for removal of chlorinated ethylenes from groundwater Poster Presentation, CSWEA/AWMA Conference on the Environment, Bloomington, MN, November 20, 2003.

Report Follows

Phyto-enhanced Remediation: A wetland Treatment System for Surface Water Protection

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Executive summary
Halogenated solvents, such as dichloroethylene (DCE), present a challenging remediation problem due to their prevalence and persistence in the environment. In groundwater contamination scenarios where the source pools cannot be located/removed, there is great demand for a long-term cost effective alternative to treat the contaminant plume. Wetland treatment is an attractive alternative because of its passive nature and low operation/maintenance costs. A wetland treatment system was implemented as a remedial action to protect Lake Minnetonka from a DCE plume emanating from a former manufacturing facility in Mound, Minnesota. This work was initiated to address a lack of data regarding the role of wetland vegetation in the removal of DCE by the constructed wetland. Work conducted to date suggests that wetland vegetation did not affect the size or structure of methanotrophic bacterial communities in the field, and cometabolic oxidation of DCE by methanotrophs was not a significant fate mechanism in laboratory microcosm studies. In the case of cattails, transport from the subsurface to the atmosphere via plant tissues is the primary fate mechanism for DCE in laboratory microcosms. The transpiration stream concentration factor, the primary metric for vascular uptake of contaminants by plants, was significantly higher (~7-fold) for cattails than predicted by previously published models. This phenomenon may be attributed to volatilization/gas-phase diffusion of DCE through gas-filled voids (aerenchyma tissue) in wetland plants. Previously published models are based on terrestrial plants, such as hybrid poplar trees, which lack aerenchyma tissue. Cattails also prevented the accumulation of vinyl chloride, an anaerobic biodegradation product of DCE. Because DCE removal by cattails is strongly influenced by transpiration rate, it may be possible to adapt wetland management practices to enhance DCE removal or to moderate DCE efflux to the atmosphere if necessary.

Introduction
Halogenated solvents, such as chlorinated methanes, ethanes, and ethylenes, are among the most prevalent pollutants at contaminated sites on the National Priorities List as well as sites owned by the Department of Defense and Department of Energy. Contamination is also often observed at dry-cleaning and degreasing operations. Halogenated solvents pose an extremely difficult remediation problem. These compounds generally have low aqueous solubility and collect at impermeable layers forming pools of non-aqueous phase liquid *(1)*.

While several remediation techniques are currently available for the removal or degradation of chlorinated compounds at contaminated sites, these techniques are subject to significant technical

and economic limitations. Phytoremediation is a burgeoning technology that utilizes living plants to help remove contaminants from the environment. Phyto- and phyto-enhanced remediation are potentially low cost and aesthetically pleasing remediation alternatives. One example of phytoremediation is a wetland treatment system, in which wetland plants facilitate the removal of contaminants from water as it flows through the wetland. Wetland treatment systems are becoming widely used to treat municipal wastewater *(2-5)* as well as numerous other waste streams including landfill leachate and acid mine drainage *(6,7)*. Wetlands offer a unique remediation environment, as shown by wastewater treatment applications that take advantage of the ability of the diverse microbial population supported by the wetland environment to degrade a variety of contaminants. The root zone, or rhizosphere, of wetland plants may play an important role in supporting essential, waste-degrading microbes.

Wetland treatment systems also have great potential for removing chlorinated solvents from groundwater *(8)*. Wetlands have been shown to support microbes, such as methanotrophic bacteria, capable of degrading chlorinated solvents *(8-11)*. Wetland plants may also have the capability to take up and transpire/mineralize chlorinated solvents *(12,13)*, although this has yet to be specifically demonstrated for most wetland plants. Root systems of wetland plants may also enhance the bacterial mineralization of chlorinated solvents in the rhizosphere through the excretion of root exudates and oxygen *(14,15)*.

The objective of this research is to determine the specific roles of the soil and plants and the impact of plant-microbial interactions in the removal of chlorinated ethylenes in a constructed wetland. Additionally, this study will elucidate the effect of wetland vegetation on the growth of methanotrophic bacteria in wetland sediment.

Methods

Field Mesocosms. Three field mesocosms (one unvegetated, two planted with a mixture of cattails, giant bur-reed, bottlebrush sedge, and bulrush) were observed from April-October (the growing season for Minnesota). Porewater samples were collected from the mesocosms via stainless steel microwells embedded in the sediment at 13-cm intervals. Samples were drawn from the microwells via a glass gas-tight syringe and Teflon-lined tubing. Porewater samples were analyzed for chlorinated ethenes and methane via gas chromatography. Dissolved oxygen, sulfate, and sulfide were measured using a handheld colorimetric test kit (CHEMetrics Company Vacu-Vials™).

The effect of the root systems of wetland plants on methanotrophic biomass levels was evaluated via sampling of mesocosm sediment. Soil cores were taken from each mesocosm cell in November 2002, May 2003, and July 2003. Soil cores were split in half along the longitudinal axis, and 2-gram composite soil samples were taken at 13-cm intervals along the length of the core. These samples were stored on ice for transport to the laboratory and immediately frozen at -20°C upon their arrival. DNA was extracted from soil samples using a FastDNA spin kit for soil (Qbiogene) and methanotrophic biomass was quantified via competitive polymerase chain reaction (cPCR) focusing on 16S rRNA genes for Type I and Type II methanotrophs. Competitor DNA was prepared using 16S rRNA material from *M. methanica* (Type I) and *M. trichosporium* (Type II). Additionally, methanotrophic community structure was evaluated using nested PCR and denaturing gradient gel electrophoresis.

Laboratory Microcosms. Laboratory microcosm studies were conducted to evaluate the fate of DCE and the effect of wetland plants on methantrophic bacterial populations in a controlled system. Experimental treatments applied include: wetland plants growing in hydroponic solution and wetland plants growing in sediment from the site. Controls consisting of a glass rod in place of the wetland plant stem were included to account for any leakage through the plug. Experiments were conducted in triplicate using microcosms consisting of a root compartment and shoot compartment separated by a wax/clay composite seal. Either ¼-strength Hoagland's solution (hydroponic experiments) or synthetic groundwater with methane (plants with soil) were fed to the root compartment via flexible carboys under constant hydrostatic pressure. The air in the shoot compartment was exchanged continuously using a vacuum system. Exhaust air from the shoot compartments was passed through an activated carbon trap and two sequential potassium hydroxide traps. Replicate microcosms were spiked with a mixture of unlabeled and ^{14}C-*cis*-DCE. A new method for converting ^{14}C-TCE to ^{14}C-*cis*-DCE using Ti(III) citrate and a cobalt-porphyrin catalyst was developed as an economical alternative to purchasing commercially-synthesized ^{14}C-*cis*-DCE.

Aqueous samples (1-mL) were collected from the root compartments and analyzed for methane as well as *cis*-DCE, vinyl chloride, and ethylene were monitored using headspace analysis on a GC equipped with a flame ionization detector. A separate 0.5-mL aqueous sample was collected from the root compartment and added to a sealed 10-mL vial containing 3 mL of hexane and 1 mL of 1 M KOH solution. The vials were equilibrated overnight and the two phases were sampled separately and analyzed for ^{14}C via liquid scintillation counting (LSC) in order to determine the relative amounts of ^{14}VOC and $^{14}CO_2$ present in the root compartment solution. The activated carbon traps were extracted in hexane, and this extract was analyzed by LSC to determine the amount of ^{14}VOC transported through plant tissues. KOH traps were sampled and analyzed via LSC to determine the amount of $^{14}CO_2$ transported through the plant tissues. Transpiration was tracked by weighing the flexible carboys.

At the end of each experiment, the microcosms were dismantled, and soil was sampled for PCR and ^{14}C analysis. The plant roots were gently rinsed with DI water, and blotted dry. The plants were then divided into root, submerged shoot, and emergent shoot sections. Each section was weighed, flash-frozen in liquid nitrogen, and stored in Teflon-capped glass jars at -20 °C. DNA from triplicate soil samples was extracted as previously described and subjected to the aforementioned cPCR and nested PCR analyses. Additionally, DNA was extracted from the frozen/pulverized root tissue and subjected to the PCR analyses.

Data Analysis. To characterize the transport of chlorinated VOCs through the plants, the transpiration stream concentration factor (TSCF) was computed for each of the vegetated microcosms. TSCF is defined as follows *(6)*:

TSCF = Concentration in the transpiration stream/Concentration in bulk solution

TSCF was determined with a finite difference model utilizing the following equation:

$$Uptake_{t1\text{-}t2} = TSCF \times Trans_{t1\text{-}t2} \times (C_{bulk\ solution,\ t1} - C_{bulk\ solution,\ t2})/2$$

Variables were defined as follows:

$Uptake_{t1\text{-}t2}$ = the amount of ^{14}C trapped on the activated carbon over a specific time period
$Trans_{t1\text{-}t2}$ = the volume of water transpired over a specific time period
$C_{bulk\ solution,\ t1}$ = the concentration of ^{14}C VOCs in bulk solution at the beginning of the time period
$C_{bulk\ solution,\ t2}$ = the concentration of ^{14}C VOCs in bulk solution at the end of the time period

Uptake values were plotted versus the corresponding value of $Trans_{t1\text{-}t2} \times (C_{bulk\ solution,\ t1} - C_{bulk\ solution,\ t2})/2$ and a linear regression was performed on the data. The slope of the best fit line corresponds to the TSCF.

Results to date

Field Mesocosms. Chlorinated ethylenes were not detected at any depth in any of the mesocosms. Data provided by Barr Engineering Company indicated that both DCE and vinyl chloride were present in substantial amounts in the deeper aquifer (15-20 ft). Additionally, the vertical groundwater gradient in the vicinity of the mesocosms was neutral, indicating minimal influence of groundwater discharge on subsurface conditions in the mesocosms. Large amounts of methane were detected in all three mesocosms, with methane concentration increasing with depth in each mesocosm. While methane concentration profiles were similar for the vegetated and unvegetated mesocosms during the Spring and Fall, vegetated mesocosms exhibited depressed methane concentrations in the upper 30 cm during the height of the growing season.

While large quantities of both Type I and Type II methanotrophic bacteria were detected in all three mesocosms, no trends in population size were observed with respect to time of year, depth, or presence/absence of vegetation. The qualitative analysis of methanotroph population structure revealed the following seasonal population shifts for Type I methanotrophs: appearance of *Methylocaldum* sp. in Fall, appearance of *Methylobacter* sp. in Spring, and appearance of *Methylomonas* sp. in Summer. No trends in Type I methanotrophic population structure were observed with respect to depth or presence/absence of vegetation. No trends in Type II methanotrophic population structure was observed with respect to time of year, depth, or presence/absence of vegetation.

Laboratory Microcosms. DCE disappeared in the root compartments of microcosms with cattails under hydroponic conditions and in microcosms with soil (Figure 1). While the unvegetated hydroponic controls showed minimal loss of DCE, some DCE loss was observed in soil controls. Most of the radiolabel (60%-80%) was recovered on the activated carbon (Figure 2), indicating that transport through plant tissues was the most important fate mechanism. No $^{14}CO_2$ was observed in either the root compartment or the KOH traps. While the extent to which $^{14}CO_2$ may have been sequestered by the plant during photosynthesis is unknown, the radiation balance suggests that cometabolic oxidation of DCE by methanotrophic bacteria could account for no more than 10% of the DCE removed.

Reductive dechlorination, indicated by the appearance of vinyl chloride in the root compartment, was observed in microcosms with soil. While significant amounts of vinyl chloride accumulated in the unvegetated soil controls, vinyl chloride appearance was transient in the microcosms with cattails. It is unclear whether this difference in vinyl chloride concentration is the result of

transport of vinyl chloride through plant tissues, modification of sediment redox conditions by the plant, or cometabolic oxidation of vinyl chloride by methanotrophic bacteria.

TSCF values computed for the cattail microcosms were similar for hydroponic and soil-filled root compartments. TSCF values ranged from 2.7 to 5.1. The predicted TSCF for DCE based on log Kow is 0.75 (6), much lower than observed in this work. This suggests that another mechanism in addition to uptake in the transpiration stream is involved in translocation of DCE through plant tissues. This mechanism is likely volatilization/gas phase diffusion through gas-filled voids in the plant (aerenchyma tissue).

Ongoing work

Current work is focusing on evaluating effect of cattails on the size and structure of methanotrophic bacterial communities in the sediment from the microcosms. Additionally, analysis of plant tissues for ^{14}C content has yet to be conducted. The microcosm studies are also being repeated with giant bur-reed to determine if a different plant species will exhibit a different effect on the fate of DCE and methanotrophic bacterial populations in the wetland microcosms.

Summary of findings

While wetland plants do not appear to significantly affect the size or structure of the methanotrophic bacterial populations in a constructed wetland, they can play a significant role in removal of DCE from groundwater via vascular uptake/volatilization through tissues. Wetland plants can also prevent accumulation of the undesirable daughter product vinyl chloride. Removal of DCE by wetland plants is strongly influenced by transpiration rate, suggesting that management practices could be adapted to balance DCE removal with efflux to the atmosphere.

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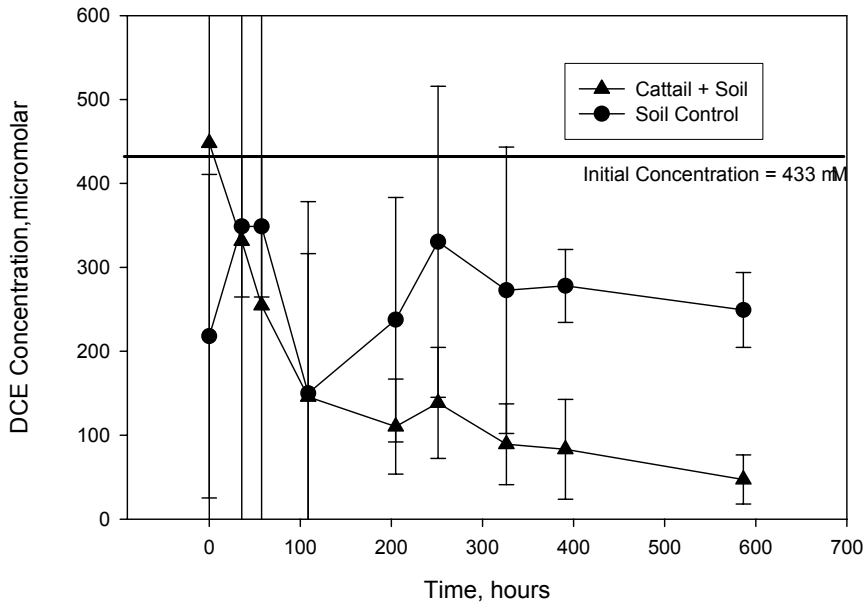

Figure 1: DCE Concentration in Root Compartments of Cattail Microcosms and Soil Controls

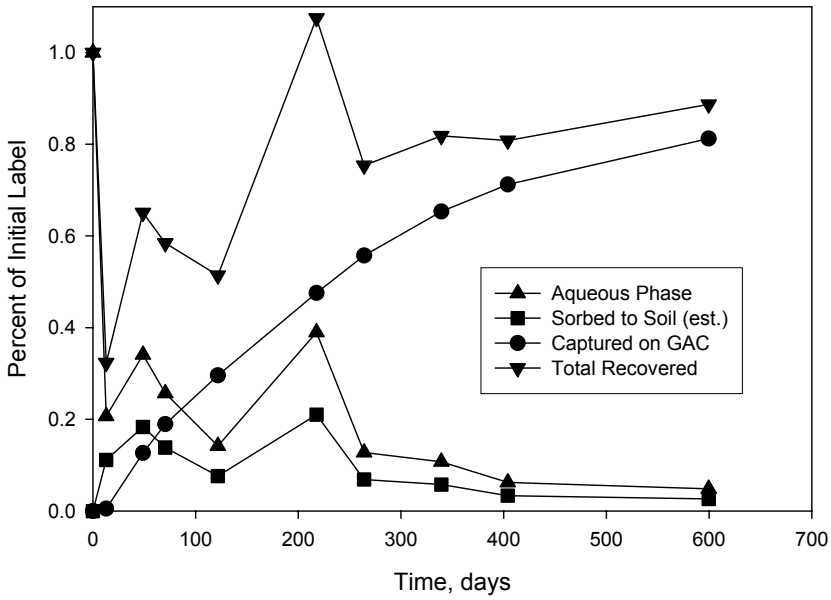

Figure 2: Activity Balance for Cattail Microcosms